PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 000595-0052	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/CA2004/001968	International filing date *(day/month/year)* 15 November 2004 (15-11-2004)	Priority date *(day/month/year)* 13 November 2003 (13-11-2003)

International Patent Classification (IPC) or national classification and IPC
IPC: A61K 35/74 ***(2006.01)***, A61K 38/00 ***(2006.01)***, A61P 35/00 ***(2006.01)***

Applicant
BIO-K PLUS INTERNATIONAL INC. ET AL

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. [X] *(sent to the applicant and to the International Bureau)* a total of 6 sheets, as follows:
      [X] sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      [ ] sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. 1 and the Supplemental Box.
   b. [ ] *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s))
      , containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - [X] Box No. I Basis of the report
   - [ ] Box No. II Priority
   - [X] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - [ ] Box No. IV Lack of unity of invention
   - [X] Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - [ ] Box No. VI Certain documents cited
   - [ ] Box No. VII Certain defects in the international application
   - [ ] Box No. VIII Certain observations on the international application

Date of submission of the demand 13 June 2005 (13-06-2005)	Date of completion of this report 6 March 2006 (06-03-2006)
Name and mailing address of the IPEA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001(819)953-2476	Authorized officer Cynthia Bruce-Payne (819)997-4921

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/CA2004/001968

Box No. I Basis of the report

1. With regard to the **language**, this report is based on:

[X] the international application in the language in which it was filed

[ ] a translation of the international application into , which is the language of a translation furnished for the purposes of:

[ ] international search (Rules 12.3(a) and 23.1(b))

[ ] publication of the international application (Rule 12.4(a))

[ ] international preliminary examination (Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*

[ ] the international application as originally filed/furnished

[X] the description:

[X] pages 2-15 as originally filed/furnished

[X] pages* 1, 16, 17 received by this Authority on 14 November 2005

[ ] pages* received by this Authority on

[X] the claims:

[ ] pages as originally filed/furnished

[ ] pages* as amended (together with any statement) under Article 19

[X] pages* 18-20 received by this Authority on 14 November 2005

[ ] pages* received by this Authority on

[ ] the drawings:

[ ] pages as originally filed/furnished

[ ] pages* received by this Authority on

[ ] pages* received by this Authority on

[ ] a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. [X] The amendments have resulted in the cancellation of:

[X] the description, pages 1, 16, 17 as originally filed

[X] the claims, Nos. 24-31 as originally filed

[ ] the drawings, sheets/figs

[ ] the sequence listing *(specify):*

[ ] any table(s) related to sequence listing *(specify):*

4. [ ] This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

[ ] the description, pages

[ ] the claims, Nos.

[ ] the drawings, sheets/figs

[ ] the sequence listing *(specify):*

[ ] any table(s) related to sequence listing *(specify):*

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The question whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

[ ] the entire international application

[X] claims Nos. 19-23

because:

[X] the said international application, or the said claims Nos. 19-23
relate to the following subject matter which does not require an international preliminary examination *(specify):*

Although claims 19 to 23 encompass a method of treatment of the human/animal body which this Authority is not required to examine under Rule 67.1 (iv) of the PCT, the international preliminary report on patentability has been established on the basis of the alleged effects of the compositions or supernatants referred to therein.

[ ] the description, claims or drawings *(indicate particular elements below)* or said claims Nos.
are so unclear that no meaningful opinion could be formed *(specify):*

[ ] the claims, or said claims Nos. are so inadequately supported
by the description that no meaningful opinion could be formed *(specify):*

[ ] no international search report has been established for said claims Nos.

[ ] a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

[ ] furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

[ ] furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

[ ] pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13*ter*.1(a) or (b) and 13*ter*.2.

[ ] a meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions, and such tables were not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

[ ] the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

[ ] See Supplemental Box for further details.

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1 to 4, 9 to 23	YES
	Claims	5 to 8	NO
Inventive step (IS)	Claims	None	YES
	Claims	1 to 23	NO
Industrial applicability (IA)	Claims	1 to 23	YES
	Claims	None	NO

2. Citations and explanations (Rule 70.7)

Reference is made to the following documents:
D1: WO 03/045405 A2 (LUQUET, F-M. et al.) 5 June 2003
D2: ARIMOCHI, H. et al. BIOCHEM BIOPHYS RES COMMUN. 1997, Vol. 238, No.3, pages 753-757
D4: MITAL, B.K. et al. CRIT REV MICROBIOL. 1995, Vol.21, No.3, pages 175-214
D5: MATSUZAKI, T. INT J FOOD MICROBIOL. 1998, Vol. 41, No.2, pages 133-140
D6: GRIFFIOEN, A.W. et al. PHARMACOL REV. 2000, Vol.52, No.2, pages 237-268

Document D1 discloses lactic compositions comprising bacterial strains *Lactobacillus acidophilus*, including strain I-1492, in combination with *Lactobacillus casei*. In addition, the disclosed lactic compositions in combination with a chemotherapeutic agent are useful in the prevention or treatment of cancer, i.e. an angiogenesis dependent disorder. Further, D1 discloses a supernatant obtained from the mixed lactic composition and determined that said supernatant does not induce apoptosis in cancer cells.

Document D2 discloses the use of an animal model to demonstrate the *in vivo* inhibition of aberrant crypt foci, correlated with precursor lesions of colon cancer, by cultures of *Lactobacillus acidophilus* as well as supernatants derived therefrom. Further, the culture supernatants were obtained by centrifugation of *Lactobacillus acidophilus* cultures and then sterilized by filtration through a filter with a pore size of 0.45 m (see page 754, left col). In addition, the observed inhibition of tumor progression was attributed to unidentified substances within the supernatant derived from the *Lactobacillus acidophilus* cultures.

Document D4 is a review article which discloses the health benefits associated with consumption of lactic compositions containing *Lactobacillus acidophilus* and summarizes numerous *in vitro* and *in vivo* studies. In particular, document D4 recapitulates the known anti-carcinogenic, hypocholesterolemic and antagonistic activities against intestinal pathogens of *Lactobacillus acidophilus*.

Document D5 is a review article which discloses the anti-tumour, anti-metastatic and immunomodulatory activity of *Lactobacillus casei* both *in vitro* and *in vivo*.

Continued: see Supplemental Box

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation Box V, 2. Citations and Explanations

Document D6 is a review article dedicated to the subject of angiogenesis. In addition to the molecular pathways associated with physiological angiogenesis document, D6 reviews aspects of pathological angiogenesis as a target to treat or prevent diseases associated with excessive angiogeneiss, such as, cancer and chronic inflammation e.g. rheumatoid arthritis.

Novelty and Inventive Step - Articles 33(2) and 33(3) PCT

The problem to be solved by the instant application is the provision of a nutraceutical product that has antiangiogenic properties to be used in the prevention or treatment of an angiogenesis dependent disorder, such as, cancer. The disclosed nutraceutical formulations are lactic compositions comprising a mixture of bacterial strains *Lactobacillus acidophilus* and *Lactobacillus casei*. Alternatively, the lactic compositions are cell-free supernatants derived from the conditioned media of the mixed *Lactobacillus* cultures. The present application discloses that the cell-free supernatants inhibit formation of capillary structures *in vitro* and the VEGF-induced migration of endothelial cells *in vitro*.

Document D1 is considered to be prejudicial to the novelty of claims 5 to 8. Notably, D1 does not disclose the characterization of any antiangiogenic property inherent is said supernatant. However, in the absence of any specified use, recitation that the claimed product, i.e. supernatant, is "characterized" by an inherent property does not reinstate patentability to an old and known product. Consequently, the instantly claimed supernatant falls within the scope of the supernatant disclosed in D1, claims 5 to 8 are thus, not compliant with article 33(2) PCT.

In the letter received by this Authority on 11 November 2005 applicant has argued in favour of the inventiveness of claims 1 to 4, and 9 to 23. In particular, applicant alleges that the prior art would not have directly led a skilled person to the compositions, supernatant and claimed uses thereof without undue experimentation because the prior art does not suggest a "relationship between *Lactobacillus acidophilus* and *Lactobacillus casei* and their broth with angiogenesis, nor do they suggest or teach a method of preventing or treating angiogenesis dependent disorders by using *Lactobacillus acidophilus* and *Lactobacillus casei* and their broth." The examiner respectfully disagrees.

None of the cited prior art documents alone explicitly disclose the use of a lactic composition comprising a mixture of *Lactobacillus acidophilus* and *Lactobacillus casei* or a supernatant derived therefrom as an antiangiogenic agent. However, in view of document D2 or D4 taken with document D5 in combination with document D6 an inventive step is not acknowledged under Article 33(3) PCT for the subject matter set forth in claims 1 to 4, and 9 to 23.

Firstly, the cited art does teach that lactic compositions do limit or eliminate tumour progression and it is suggested that they already exhibit antiangiogenic properties because they possess anti-tumour and anti-metastatic characteristics, for instance as outlined in either D2, D4 or D5. Secondly, D2 discloses that *Lactobacillus acidophilus* culture supernatants contain unidentified anti-tumourigenic substances. Moreover, the prior art demonstrated the anti-tumour effects of lactic compositions using a variety of *in vivo* animal models. In contrast, the alleged invention discloses *in vitro* data which looked at end-points with respect to formation of capilalry structures by HUVECs and migration assays in response to VEGF.

Continued: see Supplemental Box

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation Box V, 2. Citations and Explanations

Notably, the cited prior art acknowledges that the precise molecular mechanisms, by which *Lactobacillus casei*, *Lactobacillus acidophilus* and/or *Lactobacillus acidophilus* supernatant exert anti-tumour and anti-metastatic effects have not been fully elucidated. Nevertheless, it is well established that *Lactobacillus* compositions and/or their supernatants are useful in the prevention and treatment of disorders which are associated with pathological angiogenesis, in particular, cancer. Further, a skilled person would appreciate that anti-metastatic acitivity is correlated with the ability to act as an antiangiogenic agent, for example, see D6. Therefore, the cited prior art would have guided a skilled person to prepare a mixed composition comprising *Lactobacillus casei*, as taught in D5, and *Lactobacillus acidophilus*, as taught in D2 or D4, along with their broth or cell-free supernatants derived therefrom with a reasonable expectation that such a composition would be useful for the prevention or treatment of an angiogenesis dependant disorder, in particular, cancer, without the exercise of an inventive step. Further, applicant has not demonstrated any improvement, advantage or unexpected effect of the use of a combination of *Lactobacillus casei*, *Lactobacillus acidophilus* along with their broth or the use of mixed cell-free supernatants as instantly claimed. Accordingly, the ability of a mixed lactic composition comprising *Lactobacillus casei*, *Lactobacillus acidophilus* along with their broth or cell-free supernatants derived therefrom to act as an antiagiogenic agent does not appear to impart any distinguishing technical characteritics which could be used to distinguish the claimed uses from those disclosed in documents D2, D4, or D5 which all pertain to the treatment and prevention of an angiogenesis dependent disorder.

Industrial Applicability - Artricle 33(4) PCT

Claims 1 to 18 appear to define subject matter that has industrial applicability under Article 33(4) of the PCT, based on the function of the lactic composition or supernatants derived therefrom of the instant application.

For the assessment of claims 19 to 23 on the question of whether or not they define subject matter that has industrial applicability, no unified criteria exists in the PCT. Further, the patentability of said claims can depend upon their formulation. Although the methods per se defined in claims 19 to 23 relate to subject matter which this Authority is not obliged to examine under Rule 67.1 (iv) of the PCT, the use of the compounds referred to therein for the preventiono r treatment of an angiogenesis dependent disorder appears to represent subject matter that has industrial applicability.

USE OF STRAINS OF LACTOBACILLUS AND BYPRODUCTS THEREOF FOR INHIBITING FORMATION OF BLOOD VESSELS

FIELD OF THE INVENTION

The present invention is related to the field of probiotics useful in the prevention of disorders.

BRIEF DESCRIPTION OF THE PRIOR ART

Cancer research aims to discover means by which the aggressive growth of solid tumours and their metastases can be abolished in a specific way without causing treatment resistance, or provoke excessive toxicity in treated patients. The challenge is high, since the transformation of normal cells into tumour cells is associated with the acquisition of resistance to most cytotoxic agents presently used in therapy. Several studies done in the last few years have demonstrated that tumour cells do not represent the only factor responsible for tumour growth. Blood vessels present within these tumours play also a crucial role. It has been clearly established that blood vessels, formed by the angiogenesis process (Figures 15 and 16), are essential to aggressive growth of tumours and their metastases. This angiogenesis is due to the capacity of tumour cells to secrete a certain number of angiogenic factors, like vascular endothelium growth factor (VEGF) and fibroblastic growth factor (FGF), linking with high affinity the surface of endothelial cells. The stimulation of endothelial cells by these factors, results not only in an increase of secretion of enzymes degrading the extra cellular matrix components, but also in the stimulation of the migration and the proliferation of these cells. The thus stimulated cells invade the matrix surrounding the tumours, forming a capillary network which will ensure the growth of tumour cells, by giving them nutrients and oxygen necessary for their development. The inhibition of blood contribution to the tumours constitutes thus a target of choice for the development of new therapeutic anticancerous approaches targeting specifically angiogenesis to limit or eliminate tumour progression.

It is estimated that life habits and eating habits are responsible for more that one third of new diagnosed cancers. Consequently, prevention (Nutra-prevention) presently creates a big interest and it is estimated that in the following few years, it will bring reduction in

1. Daoy (brain medulloblastoma)
2. U-87 (brain glioblastoma-astrocytoma)
3. Jurkat (leukemia lymphocytes)

These studies will permit to better characterize and identify new molecular targets, modulated by BIO-K-Plus, endothelial cells and cancerous cells modulated by BIO-K-Plus.

1.1 Preparation of study material

In the present study, the inventors have characterized the supernatants action of irradiated lactic acid bacteria at 3 kGy (S3), 6 kGy (S6) and 9 kGy (S9). These supernatants have been obtained after two centrifugations (one at 6 000g for 15 min at 4°C and the other at 10 000g for 20 min at 4°C). They have then been filtered on two filters (on filter of 0.05 µm followed by a filter of 0.22 µm) to obtain bacteria free sterile supernatants and in order to be able to treat divers cells lines. Supernatants have been kept at -80°C until use.

For those studies, the inventors have used a concentration of supernatants equivalent to 10^8 bacteria, since it is at this concentration that the inhibitor effect is maximal.

1.2 *In vitro* characterization of antiangiogenic properties of BIO-K-Plus on HUVECs.

The inventors have verified if bacterial supernatants have an effect on endothelial cells. The WST-1 technique, which measures mitochondrial activity of cells, has permitted the study of the cell proliferation of HUVECs. The supernatants did not seem to have an inhibitory effect on the cells proliferation (Figures 8 and 9; n=2). The inventors have then evaluated the migratory potential of cells in presence of bacterial supernatants and the results have been positive. The inventors have verified if supernatants inhibit the stimulation of HUVECs migration on gelatine induce by VEGF, the mitogen the most often associated with angiogenesis phenomena. The supernatants inhibit completely the migration by VEGF but also the basal level of migration at approximately 50% (Figure 10B). The inhibitor effect of supernatants does not seem to be specific to VEGF. The assays of tube formation on Matrigel (in laminin rich matrix, reconstituting the basal

membrane and which permits the endothelial cells differentiation in similar structures to capillary blood vessels) demonstrate that bacterial supernatants inhibit in a significant way the tube formation compared to a control in HUVECs (Figures 11A and11B; n=2). These results indicate that bacterial supernatants contain molecules which have an antiangiogenic potential.

1.3 Conclusion

The inventors have demonstrated that the supernatants coming from lactic acid bacteria containing *Lactobacillus acidophilus* and *Lactobacillus casei* has an antiangiogenic activity.

CLAIMS

1. A lactic composition comprising a mixture of bacterial strain, *Lactobacillus acidophilus and Lactobacillus casei*, and a whole broth of said mixture, characterized in that the lactic composition is for the prevention or the treatment of angiogenesis dependant disorders.

2. A lactic composition according to claim 1, characterized in that the at least one *Lactobacillus acidophilus* strain is strain I-1492 deposited at the CNCM.

3. A lactic composition according to claim 2, characterized in that it comprises at least 500 millions per gram of a population of living and active micro-organisms of the *Lactobacillus acidophilus* strains after 90 days under refrigeration, where at least 380 millions per gram are micro-organisms of the *Lactobacillus acidophilus* CNCM I-1492 strain.

4. A lactic composition according to claim 3, characterized in that it further comprises fermented milk proteins or fermented soy proteins.

5. A supernatant obtained from the lactic composition as defined in any one of claims 1 to 4, characterized in that said supernatant exhibits antiangiogenic properties.

6. The supernatant according to claim 5, characterized in that said supernatant is concentrated.

7. The supernatant according to claims 5 or 6, characterized in that said supernatant is 10X concentrated.

8. The supernatant according to any one of claims 5 to 7, characterized in that it comprises molecules of a size larger than 5000 kDa.

AMENDED CLAIMS

9. Use of the supernatant as defined in any one of claims 5 to 8, as an antiangiogenic agent.

10. Use of the supernatant as defined in any one of claims 5 to 8, in the prevention or the treatment of an angiogenesis dependant disorder in a mammal.

11. Use according to claim 10, wherein said mammal is a human being.

12. Use according to claim 10, wherein said disorder is selected from the group consisting of retinopathy, infantile haemangioma, rheumatoid arthritis, psoriasis, duodenal ulcers, post-angioplasty restenosis and tumour growth.

13. Use of a supernatant according to claim 12, wherein said disorder is tumour growth.

14. Use of the lactic composition as defined in any one of claims 1 to 4, as an antiangiogenic agent.

15. Use of the lactic composition as defined in any one of claims 1 to 4, in the prevention or the treatment of an angiogenesis dependant disorder in an mammal.

16. Use according to claim 15, wherein said mammal is a human being.

17. Use according to claim 15, wherein said disorder is selected from the group consisting of retinopathy, infantile haemangioma, rheumatoid arthritis, psoriasis, duodenal ulcers, post-angioplasty restenosis and tumour growth.

18. Use according to claim 17, wherein said disorder is tumour growth.

19. Method for prevention or treatment of an angiogenesis dependant disorder, the method comprising the step of administering to a mammal an effective

AMENDED CLAIMS

amount of the lactic composition as defined in anyone of claims 1 to 4 or of the supernatant as defined in any one of claims 5 to 8.

20. Method according to claim 19, wherein said mammal is a human being.

21. Method according to claim 19, wherein said disorder is selected from the group consisting of retinopathy, infantile haemangioma, rheumatoid arthritis, psoriasis, duodenal ulcers, post-angioplasty restenosis and tumour growth.

22. Method according to claim 21, wherein said disorder is tumour growth.

23. Method according to any one of claims 19 to 22, wherein said administration is oral administration.

AMENDED CLAIMS